II. REMARKS

Reconsideration of the above-identified application in view of the amendments above and the remarks following is respectfully requested.

Before the amendments made herein, claims 44, 46, 52-57, 59, 60 and 63-74 were pending and under examination. Claims 44, 46, 52-57, 59, 60, 63, and 65-74 have been rejected. Claim 64 has been objected to. Claims 57 and 71 have now been cancelled without prejudice. Claims 44, 52, 63, 64, 65, 66 and 74 have now been amended. New claims 75-80 have now been added. Accordingly, after the amendments made herein are entered, claims 44, 46, 52-56, 59, 60, 63-70 and 72-80 will be pending.

Rejection under 35 U.S.C. § 103

Claims 44, 46, 52-57, 59, 60, and claims 63 and 66-74 are rejected under 35 U.S.C §103 as allegedly unpatentable over Kuby et al. in view of Aridor et al. and U.S. Pat. No. 5,807,746 to Lin. The Examiner's rejections are respectfully traversed below. Please note that claims 57 and 71 have been cancelled herein without prejudice, claims 44, 52, 63, 64, 65, 66 and 74 have been amended and new claims 75-80 have been added.

More particularly, the Examiner alleges that Kuby discloses that the inhibition of mast cell degranulation is a known mechanism to treat allergies. The Examiner further alleges that Aridor teaches the use of two peptides, KNNLKECGLY and KENLKDCGLF, in inhibiting mast cell degranulation when given to permeabilized cells *in vitro*. Lastly, the Examiner alleges that Lin teaches adding the sequence AAVALLPAVLLALLAP to any known biologically active peptide allows transportation of the active peptide into the cell for *in vivo* therapy. The Examiner concludes that one of ordinary skill in the art would be motivated to add the importation peptides of Lin to the peptides taught by Aridor to treat allergies, and that one of ordinary skill in the art would have a reasonable expectation of success in producing the claimed invention.

In response, Applicants respectively submit that the prior art would not motivate the ordinary skilled artisan to attach the hydrophobic signal peptide

disclosed by Lin to the inhibitory peptides taught by Aridor for the following reasons, as discussed in detail below.

First, one would not have been motivated to select the peptide of Lin because it belongs to a class of signal peptides that is relatively obscure and poorly understood (see Section I below). Second, it turns out that the C-terminal proline of this signal peptide is crucial, and that this feature appears in less that 10% of the known signal peptides, making the invention further unobvious (see Section II below). Third, the art makes clear that this field is not predictable and must be assessed on a case-by-case basis. Thus, at best, the complexes of the subject invention were obvious to try, which is not sufficient for a finding of obviousness (see Section III below).

In addition, even if a *prima facie* finding of obviousness could be made (which it cannot for the reasons stated above), such a finding is rebutted by a clear showing of a) unexpected results (see Section IV below) and b) a long-felt and unmet urgent need (see Section V below).

I. One of Ordinary Skill in the Art would not have been motivated to select the particular importation peptide taught by Lin from the myriad of peptides which were available at the time of filing of the present invention

Naturally occurring peptides and proteins which penetrate cell membranes have been known in the art since the early 1990s. Members of this group of peptides utilize non-endocytic translocation approaches to penetrate cells via a receptor independent pathway. See, for example, Scheller et al., *Eur. J. Biochem.*, 267:6043-6050 (2000). At the time of the present invention, more than a hundred such peptides and a few proteins were known.

From the state of the cell importation art at the time of the present invention, it is quite clear that the importation (also referred to herein as penetrating or transduction) peptide utilized by the present inventors would have been overlooked or discounted by the skilled artisan. This is because the transduction peptide which forms a part of the presently claimed complex belongs to **the less studied and relatively obscure hydrophobic class of transduction peptides**. By contrast, the 'amphipathic' peptides, described below, clearly represent the most abundant and functionally proven class of transduction peptides.

Knowledge gained from studies of transduction peptides led researchers to design amphipathic peptides, which rely upon electrostatic interactions for cell penetration. As a result, reports on transduction peptides (also known as "cell penetrating peptides") which rely upon such non-endocytic interactions for cell penetration represent **well over 80%** of the publications in the field of protein transduction (see Figure 1 of enclosed Appendix 1). In addition, synthetic amphipathic peptides designed for membrane penetration are widely considered the most efficient in vivo carriers for intra-cell delivery of molecules. Indeed, two important reviews in the field of intra-cell molecule delivery teach the abundance of these peptides and their suitability for intra-cell delivery of attached compounds and peptides. Vives, E., *Journal of Controlled Release*, 109:77–85 (2005); and Zorko and Langel, *Advanced Drug Delivery Reviews*, 57:529– 545 (2005).

Thus, even at the time of the subject invention, amphipathic peptides were by far the most well known and documented class. Moreover, their mechanism of action in penetrating cells was well established and mimicked that of the naturally occurring amphipathic peptides. Accordingly, at the time of the present invention, the skilled artisan would have been strongly inclined to select a signal peptide from this group of transduction peptides in designing a mast cell degranulation inhibiting complex.

In contrast, the transduction peptide which forms a part of the presently claimed complex belongs to the less studied and relatively obscure hydrophobic class of transduction peptides. One reason why the skilled artisan would not select a peptide from this hydrophobic class is because of its counter-intuitive and mysterious nature.

More specifically, hydrophobic cell penetrating peptides are derived from signal peptide sequences which, in their native form, function to direct newly synthesized proteins to the membrane or for secretion out of the cell, a role directly opposite to the function sought from cell penetration peptides. Indeed, the exact mechanism of cell penetration for signal sequence-derived peptides has yet to be elucidated. As a result, this class of cell penetrating peptides is yet to gain acceptance and be in wide spread use, and this was even more so the case at the time of the subject invention. See, for example, Hawiger, J., *Current Opinion in Chemical Biology*, 3:89–94 (1999). As a result, very few members of this class of cell penetrating peptides have been successfully utilized to date. Indeed only a small

percentage hydrophobic peptides with proven cell penetrating function are presently known in the art compared to those in the more well known and better understood amphipathic class.

Thus, it is quite clear that was not an ordinary but rather an extraordinary, and certainly unobvious, decision on the part of the present inventors to test the signal peptide which forms a part of the presently claimed complex.

II. A unique structural feature of the claimed complex molecule mediates its inhibitory activity

To further illustrate the unobviousness of the hydrophobic signal peptide of the present invention, after the date of the present invention, the subject inventors discovered why this peptide was successful while so many others failed. It turns out that a unique sequence feature of this hydrophobic transduction peptide was responsible for the successful importation and subsequent degranulation inactivation, specifically a C-terminal proline. Indeed, this crucial discovery merited the filing of another family of patent applications (see, for example, U.S. Pat. Application No. 10/465,826).

This C-terminal proline feature is present **in less than 10%** of known cell penetrating peptides (see Appendix 1). Thus, given the remote chance that the ordinary skilled artisan selects a hydrophobic cell penetrating peptide (see Section I above), it is **more than ten times more remote** that the selected peptide would include a C-terminal proline, which is crucial for intra-cell activity.

III. Undue Experimentation

In addition, the present complex was shown to be effective in inhibiting mast cell degranulation both *in-vitro* and more importantly ***in-vivo***. This result could not be predicted and would have required, as of the date of the subject invention, undue experimentation.

For example, Jones et al. (*Biochemica et Biophysica Acta*, 1745:207-214 (2005), enclosed herewith) reported effective intra-cell delivery of a decapeptide inherently having mast cell degranulation inhibition effect linked to a cell penetrating peptide. However, following cell penetration, the complex described by Jones induced

the dual phosphorylation of p42/p44 MAP kinases similarly to the known secretagogue mastoparan (MP). In other words, the molecule designed by Jones, while expected to inhibit mast cell degranulation, caused the opposite effect and **induced** mast cell degranulation.

The findings of Jones clearly demonstrate that, in the field of the subject invention, in the absence of an elucidated mechanism (as for example described in a subsequent filing of the instant inventors in U.S. Pat. Application No. 10/465,826) one must evaluate the effect of a cell penetrating peptide on intra-cell delivery and intra-cellular function of the inhibitory peptide on a case by case basis. More importantly, absent clear and convincing guidance, Jones teaches that the success of the combination of a cell penetrating peptide and an inhibitory peptide is not obvious. Certainly, there was no such guidance regarding the claimed combination of the cell penetrating peptide and an inhibitory peptide of the subject invention at the time the invention was made.

IV. The claimed peptide complexes are effective in inhibiting both IgE dependent and IgE independent mast cell degranulation, and therefore show unexpected results

Even if there were a *prima facie* case of obviousness, which as discussed above there is not, such a case would be rebutted by the clear showing of unexpected results of the claimed peptide complexes. See MPEP Sec. 716.02. The unexpected superiority of the claimed peptide complexes is clearly demonstrated in their ability to inhibit **both** IgE dependent and IgE independent allergic conditions. In contrast the inhibitory peptide taught by Aridor was shown only to inhibit the receptor independent (IgE independent) positively charged secretagogue compound 48/80. In other words, Aridor does not teach or remotely suggest that its inhibitory peptide can be effective against any IgE dependent conditions.

For example, *in-vivo* results disclosed in the subject application (see Example 4, pages 27-35), as well as results obtained following filing of the subject invention using the teachings of the present invention (see attached Appendix 2) show an unprecedented efficacy of the claimed peptide complexes in treating a variety of mast cell associated disorders including skin allergy (IgE independent compound 48/80

induced, see section A of Appendix 2), ophthalmic allergy (both IgE independent-compound 48/80 induced; and IgE dependent-ragweed pollen induced, see sections B and C of Appendix 2) and asthma (ovalbumin induced IgE-dependent, see section D of Appendix 2).

In addition, treatment of skin allergies with the peptide complex was as effective as the gold standard Cromoglycate and more potent than Fenistil Gel and Ceterizine (see section A of Appendix 2). This peptide also effectively blocked IgE-independent conjunctivitis, showing similar efficacy as steroids and two-fold more efficacy than cromoglycate (see section B of Appendix 2). Similar results were obtained in IgE-dependent conjunctivitis, wherein treatment was as effective as with commercially available drugs (see section C of Appendix 2). In a rat model for asthma, treatment with the peptide significantly reduced bronchoconstriction (see section D of Appendix 2).

Thus, these unexpected results rebut any case of obviousness alleged against the peptide complexes of the present invention. While Aridor demonstrated an efficacy of the decapeptide in inhibiting IgE-independent mast cell degranulation, it failed to teach any effect against IgE-dependent mast cell degranulation, which can only be demonstrated *in vivo*.

More importantly, at the time of the subject invention, IgE-independent mast cell degranulation and IgE-dependent mast cell degranulation were thought to occur via separate mechanisms or pathways. Thus, Aridor does not remotely suggest the unexpected results of the subject invention.

V. Long-felt need

Any argument of obviousness of the claimed invention is also rebutted by a long-felt and unmet need. See MPEP sec. 716.04. Thus, the non-obviousness of the subject invention is supported by a lack of any prior art publication which teaches the present invention in the six year period that the subject invention is alleged to be obvious. Numerous research groups have been actively researching allergy and specifically mast cell degranulation. In fact, to illustrate the urgency of this need, a cursory search through Medline for publications which address mast cell degranulation revealed no less than **3000** articles and hundreds of reviews. Moreover,

the fact that Aridor's findings were published in *Science*, one of the most important scientific publications in the world, further supports the importance of this field.

However, in the **six years** spanning between the publication date of Aridor and filing of the present invention no evidence of successful cell importation of the peptides described by Aridor was taught or suggested in any publication. If the prior art cited by the Examiner provided the motivation and tools necessary to make the present invention, publication of results would have been likely during this time period since, clearly, there has been a long felt and urgent need for novel allergy treatments.

Summary

In summary, the prior art would not have motivated the skilled artisan to attach the hydrophobic signal peptide disclosed by Lin to the inhibitory peptides taught by Aridor. First, one would not have been motivated to select the peptide of Lin because it belongs to a class of signal peptides that is relatively obscure and poorly understood. Second, the C-terminal proline of this signal peptide is crucial, and that this feature is present in less that 10% of the known signal peptides, making the invention further unobvious. Third, the art makes clear that this field is not predictable and peptide complexes must be assessed on a case-by-case basis. Thus, the complexes of the subject invention were, at best, obvious to try, which is not sufficient for a finding of obviousness.

In addition, even if a finding of obviousness could be made (which it cannot for the reasons stated above), such a finding is rebutted by a clear showing of a) unexpected results and b) a long-felt and unmet urgent need. For all of these reasons, Applicants respectfully request that the rejection of the claims as allegedly obvious be withdrawn.

Amendments Made to the Claims and New Claims Added

All the amendments made to the claims and the new claims added herein find ample support in the specification as filed.

The limitation "preventing mast cell degranulation" finds support, for example, on page 3, lines 19-23 and page 7, lines 10-21 of the subject application.

The limitations "mast cell degranulation is IgE-dependent" and "mast cell degranulation is IgE-independent" find support, for example, on page 2, lines 24-25 and page 3, lines 1-2 and lines 18-19.

III. CONCLUSION

In view of the above amendments and remarks it is respectfully submitted that claims 44, 46, 52, 53, 54, 55, 56, 59, 60, 63, 64, 65, 66, 67, 68, 69, 70, 72-80 are now in condition for allowance. Prompt notice of allowance is respectfully and earnestly solicited.

Respectfully submitted,

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Enclosures:
Request for Continued Examination (RCE);
A Petition for Three Month Extension of Time;
Appendix 1;
Appendix 2;
Declaration Signed by Dr. Ronit Sagi-Eizenberg;
Curriculum Vitae of Dr. Ronit Sagi-Eizenberg;
Scheller, et al;
Vives;
Zorko, et al;
Hawiger;
Jones, et al